Pharmacodynamic Optimization for Treatment of Invasive Candida auris Infection

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ABSTRACT

*Candida auris* is an emerging, multidrug-resistant threat. The pharmacodynamics of three antifungal classes against nine *C. auris* strains was explored using a murine invasive candidiasis model. The total drug median PD target associated with net stasis was a fluconazole AUC/MIC of 26, amphotericin B Cmax/MIC of 0.9, and micafungin AUC/MIC of 54. The micafungin PD targets for *C. auris* were ≥20-fold lower than other *Candida* species in this animal model. Clinically relevant micafungin exposures produced the most killing among the three classes.
Candida auris is an emerging multidrug-resistant threat to human health across the globe (1, 2). The first documented clinical case of this species occurred in Japan in 2009 (3). Since then, infections due to C. auris have been published from numerous countries throughout the world (1, 2, 4-17). Unfortunately, antifungal therapeutic failure and mortality have been commonly reported. This is felt in part due to antifungal resistance. Many isolates exhibit high triazole and polyene minimum inhibitory concentrations (MICs). This might be expected as the species is phylogenetically related to C. krusei, C. lusitaneae, and C. haemulonii, which are known to be less susceptible to these antifungal classes (18, 19). Variable in vitro susceptibility results have been noted for the echinocandins (1), rendering some isolates potentially clinically resistant to all three classes of commonly used antifungal agents. The optimal antifungal agent and dosing regimen for treatment of these infections have not been defined. As such, preliminary susceptibility breakpoints are based on limited in vitro data using breakpoints developed for other Candida species.

The goal of the present studies was to define the pharmacokinetic/pharmacodynamic (PK/PD) target for the three available antifungal drug classes against this emerging pathogen. Specifically, we designed an in vivo PK/PD study to compare the treatment effects of fluconazole, micafungin, and amphotericin B using the neutropenic murine model of invasive candidiasis against nine clinical isolates of C. auris (Table). Strains were chosen to include those with variable in vitro susceptibility to available antifungal drug classes. Strains were screened for fitness in the animal model prior to treatment studies (Table). Fluconazole, micafungin and amphotericin B
deoxycholate were prepared as described in manufacturer instructions. Antifungal susceptibility testing was performed according to CLSI guidelines for fluconazole and micafungin or Etest for amphotericin B (20, 21). MIC varied by 128-fold for fluconazole (range 2 – 256 mg/L), 32-fold for micafungin (range 0.125 – 4 mg/L) and 10-fold for amphotericin B (range 0.38 – 4 mg/L). The neutropenic disseminated candidiasis model was used for all experiments. Three mice per treatment or control group were included. Mice were inoculated with 6.34 ± 0.08 log_{10} CFU/ml via the lateral tail vein with each of the nine strains. Antifungal treatment began 2 hours after inoculation and continued for 96 hours at which time mice were euthanized for CFU determination in the kidneys. Drug dosing consisted of: fluconazole 0.78, 3.125, 12.5, 50, and 200 mg/kg/12h by SC administration, micafungin 0.3125, 1.25, 5, 20, and 80 mg/kg/24h by IP administration, or amphotericin B deoxycholate 0.078, 0.3125, 1.25, 5, and 20 mg/kg/24h by IP administration. The treatment studies were designed to include clinically relevant exposures. Organism burden in mouse kidneys after 4 days (96 h) of therapy were compared to the Candida quantity at the start of therapy. The treatment results were analyzed using a sigmoidal Emax model (22). Pharmacokinetic exposures were obtained from our lab in this mouse model (23-25). The PK exposures were plotted relative to MIC and the previously defined PK/PD driver. Specifically, AUC/MIC was used for fluconazole and micafungin, and Cmax/MIC for amphotericin B (26, 27). The magnitude of the PK/PD index (AUC/MIC or Cmax/MIC) associated with net stasis and 1-log kill (when achieved) for each strain was calculated with the following equation:

\[ \log_{10} D = \log_{10} \frac{E/(E_{\text{max}} - E)}{(N + \log_{10} E_{50})}, \]

where \( E \) is the control growth for the static dose \( (D) \) and \( E + 1 \) is the control growth for the 1-log kill dose \( (D) \).
The results of the dose ranging studies with the nine C. auris isolates for each drug are shown in Figure 1A-C. Dose-dependent activity was observed with each strain. Net stasis was achieved against 7 of 9 strains for fluconazole. The two strains that did not achieve stasis over the dose range had MICs of 256 mg/L. Fluconazole therapy resulted in a 1-log kill for only one of the strains. In micafungin experiments, stasis, 1-, and 2-log kill endpoints were achieved against 8 of 9 strains. The single strain in which these endpoints were not met (B11211) had the highest micafungin MIC of the group at 4 mg/L. Finally, treatment of amphotericin B resulted in stasis with 8 of 9 strains. The single strain for which stasis was not observed with amphotericin B had an elevated MIC at 2 mg/L. However, only 3 of 9 strains achieved 1-log kill endpoints for amphotericin B. Thus, for each drug, the dose-effect against C. auris was proportional to MIC.

The degree to which MIC influences outcome in relation to pharmacokinetic exposures is the basis of PK/PD analyses. The results of these analyses are shown in Figure 1D-F. There was a strong relationship between the PK/PD parameter (AUC/MIC or Cmax/MIC) and treatment outcome for each drug (R² of 0.61 for fluconazole, 0.77 for micafungin, and 0.57 for amphotericin). The stasis and 1-log kill target exposures (for micafungin only) are shown in the Table. In the case of fluconazole, the stasis and ED₅₀ targets (data not shown) were similar at 26 and 19, respectively. These values are consistent with prior fluconazole studies against Candida species demonstrating AUC/MIC values of approximately 25 are associated with success in the animal model and in clinical studies in patients with candidemia (27).

For amphotericin B, the data for C. auris was also remarkably congruent with prior
studies which have shown stasis to occur at Cmax/MIC exposures of 1 – 2 (27). The stasis Cmax/MIC for the groups of C. auris strains in this study was near 1. In contrast, micafungin efficacy differed in comparison to prior data in the invasive candidiasis model with other Candida species. Despite elevated MICs (range 0.125 – 4 mg/L); micafungin drug exposures resulted in killing activity at relatively low drug exposures. The median total drug AUC/MIC associated with net stasis was only 53.7. Based on protein binding levels of 99.8% (28), this would translate into a free drug AUC/MIC target of 0.18. Previous micafungin studies demonstrated free-drug AUC/MIC targets of 12, 4, and 5 for C. albicans, C. glabrata, and C. parapsilosis, respectively (29). Thus, the PD targets observed for micafungin against C. auris were ≥20-fold lower than for other Candida species. 1-log kill exposures were also relatively low at total drug AUC/MIC of 131 (free drug AUC/MIC of 0.26). The reasons for enhanced efficacy observed for micafungin against C. auris compared to fluconazole and amphotericin B are unknown and an important area for future investigation.

Importantly, targets identified in this model with triazoles and echinocandins have correlated well with clinical outcomes in patients with invasive candidiasis (27). Thus, the present findings in this PK/PD study should be useful for forecasting effective treatment regimens for patients and in the development of preliminary susceptibility breakpoints. For example, a common daily dose of fluconazole in humans (400 mg) results in an AUC of nearly 400 mg*h/L (30, 31). Therefore, using an AUC/MIC target exposure of 26, the MIC ceiling for which success would be predicted is approximately 16 mg/L. Using the same approach, the MIC ceiling for amphotericin B would be 1 – 1.5 mg/L. These are similar to PK/PD based breakpoints for other Candida species and
these antifungals. Finally, a micafungin dosing regimen of 100 mg daily results in free drug exposures of approximately 0.3 – 0.4 mg*h/L (32). Using the stasis PK/PD target data for micafungin, the PK/PD breakpoint could be as high as 2 – 4 mg/L with standard dosing of 100 mg/d.

In sum, the current animal model PK/PD study suggests that echinocandins are likely to be the most efficacious drug class for most *C. auris* isolates. The results suggest traditional MIC breakpoints are likely to be relevant for fluconazole and amphotericin B as the drug exposures associated with optimal outcome were similar for *C. auris* compared to previous *Candida* species studies. However, micafungin demonstrated potent cidal effect against almost all strains with MIC <4 mg/L and the drug exposure targets (AUC/MIC) were significantly lower than other *Candida* species. Based on this data, echinocandins should be considered as first-line therapy for patients with *C. auris* infections with regimen tailoring based upon susceptibility results.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
REFERENCES


Table. Nine select *Candida auris* strains used in the studies including country of origin, antimicrobial susceptibility results, and 24-hour total drug PK/PD target exposures in the murine invasive candidiasis model.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Country of Origin</th>
<th>96 h Growth in Untreated Controls (CFU/kidney)</th>
<th>Fluconazole</th>
<th>Micafungin</th>
<th>Amphotericin B</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MIC (mg/L)</td>
<td>Stasis AUC/MIC</td>
<td>MIC (mg/L)</td>
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<tr>
<td>B11804</td>
<td>Colombia</td>
<td>2.17</td>
<td>2</td>
<td>51.2</td>
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<tr>
<td>B11801</td>
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<td>2.86</td>
<td>16</td>
<td>26.3</td>
<td>1</td>
</tr>
<tr>
<td>B11799</td>
<td>Colombia</td>
<td>2.08</td>
<td>16</td>
<td>36.3</td>
<td>2</td>
</tr>
<tr>
<td>B11221</td>
<td>South Africa</td>
<td>1.85</td>
<td>128</td>
<td>6.3</td>
<td>1</td>
</tr>
<tr>
<td>B11211</td>
<td>India</td>
<td>1.97</td>
<td>256</td>
<td>NA</td>
<td>4</td>
</tr>
<tr>
<td>B11785</td>
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<td>8</td>
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</tr>
<tr>
<td>B11220</td>
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<td>1.04</td>
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<td>5.0</td>
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<tr>
<td>B11203</td>
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<tr>
<td>B11104</td>
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<tr>
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<td>26.3</td>
<td>53.7</td>
<td>130.5</td>
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<td>Std dev</td>
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<td></td>
<td>18.5</td>
<td>87.9</td>
<td>235.3</td>
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</tbody>
</table>

NA, endpoint not achieved
Figure 1A-F. *In vivo* dose-response curves for 9 *C. auris* strains against fluconazole (A), micafungin (B), and amphotericin B (C). Each symbol represents the mean and standard deviation (error bars) of burden in the kidneys of three mice. The horizontal dashed line represents the burden at the start of therapy. The relationship between PK/PD index (AUC/MIC or Cmax/MIC) and efficacy for fluconazole (D), micafungin (E) and amphotericin B (F) are also shown. Each symbol is the mean burden from three mice and the horizontal dashed line is the burden at the start of therapy. A best-fit line based on the hill equation is shown as are the PD parameters E\text{max}, ED_{50}, slope (N), and the coefficient of determination (R^2).